Mechanism of DNA Cleavage Catalyzed by Mung Bean Nuclease

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INTRODUCTION

More than 300 enzymes require the presence of zinc ion in order to exert its metabolic activities. Among these enzymes, we can highlight the mung bean nuclease, an endonuclease that contains 334 residues of amino acids (MM = 39.000 Da), three S-S bridges and a sulfhydryl group. This enzyme promotes the cleavage of single and double stranded DNA and RNA molecules in 120 s. The impressive catalytic power of this enzyme demands both mechanistic analysis and quantitative description. In this work, several voltammetric techniques were used to investigate the mechanism of DNA cleavage catalyzed by mung bean nuclease. Initially, the interaction of zinc with a natural DNA (DNA of the lambda bacteriophage virus) and with three synthetic models of DNA (single and double stranded oligodeoxyribonucleotides) was studied by differential pulse polarography (DPP), cyclic voltammetry (CV) and anodic stripping voltammetry (ASV). The second stage of our work was dedicated to the study of the interaction of the Zn^{2+} ion with mung bean nuclease by ASV and CV.

EXPERIMENTAL

All chemicals were reagent grade and all solutions were prepared with triply distilled water from a quartz still (Quartex). DPP and ASV measurements were carried out on a 646 Metrohm Voltammetric Analyzer Processor connected to a 647 Metrohm electrochemical cell composed of a DME or HMDE (WE), an Ag/AgCl (KCl 3.0 mol L^{-1}) electrode (RE) and a platinum electrode (AE). CV measurements were performed using a PAR 173 Potentiostat/Galvanostat or a PAR 174 polarographic system connected to a PAR 175 Universal Programmer and a Houston X-Y recorder. A three electrode system with a HMDE (Metrohm) as working electrode, a reference electrode (Ag/AgCl or a SCE) and a Pt wire as the auxiliary electrode were used. Prior to voltammetric measurements, DNA and enzyme samples were exhaustive dialyzed against 0.01 mol L^{-1} EDTA and then further dialyzed against quartex water. The concentration of DNA and enzyme samples were determined by UV measurements at 260 and 280 nm. The complementary strands 1 (3'ATTAGCTAGCTGCAGCTGC5') and 2 (5'CGATCGACGTCGACGATTA3') of the double stranded oligo, each one consisting of 19 mer, were synthesized by using the phosphoramidite method. The purity of these strands was characterized by UV-Vis spectroscopy and mass spectrometry. The annealing of strands 1 and 2, which was confirmed by the construction of denaturing curves, was accomplished by using a programmable termo-controller.

RESULTS AND DISCUSSION

The formation of a stable complex between Zn^{2+} and the λ phage DNA was verified by using DPP (K_d = 3.4 x 10^{-11} mol L^{-1}) and CV (K_d = 6.7 x 10^{-11} mol L^{-1}) techniques (1). A stoichiometry rate of 23 Zn^{2+} ions, which have the function of stabilizing the double helix of the DNA by means of its interactions with the phosphate groups, per one lambda phage DNA molecule was determined for the reaction. The interaction of zinc with the oligos ARC5IIC2 (ACACCCAACGGAGA), 3'AOX1 (GCAAATGGCATTCTGACATCC) and PR1AECO (AAGAATTCGCIAAYCGTGYAAYCCIGCICAA) was investigated by means of CV with the main purpose of determining optimal experimental parameters for the synthesis of a model of double stranded DNA (oligo). The formation of a stable complex between Zn^{2+} and the double stranded oligo, with stoichiometry of 2:1, was evidenced by the decrease of the oxidation current of zinc with the addition of the oligo to the solution and the calculation of K_d (4.7 x 10^{-8} mol L^{-1}-ASV and 1.6 x 10^{-9} mol L^{-1}-CV) of the reaction. Several experimental evidences demonstrated that these zinc ions interact with the two terminal phosphate groups (5'), stabilizing the double helix of the oligo. A stoichiometry rate of 3 Zn^{2+} ions per one mung bean nuclease molecule was determined (K_{dT} = 2.5 x 10^{-8} mol L^{-1}-ASV and K_{dT} = 2.5 x 10^{-9} mol L^{-1}-CV). The experiments of cyclic voltammetry, which were accomplished in three temperatures, still showed the existence of two types of binding sites of zinc in the mung bean molecule, which were attributed to a mononuclear exposed binding site of zinc with catalytic function (Zn2) and to a inaccessible dinuclear binding site of zinc with structural functions (Zn1 and Zn3). Based on our experimental data and crystallographic results obtained for P1 nuclease (2), a concerted S_N2 mechanism, which assigns a catalytic function to the Zn2 and structural functions to Zn1 and Zn3, was proposed for the cleavage of a double stranded DNA catalyzed by the mung bean nuclease. In this mechanism, the hydrolysis of the phosphodiester bonds proceeds with inversion of configuration at the phosphorus center, i.e. through direct in line attack of a H_2O molecule, which it is activated by ionization by the Zn2, forming a pentacoordinate transition state, which is stabilized by an arginine. An aspartic acid, which is a ligand of the Zn2, helps to properly orient the nucleophile for the reaction. The ion Zn2, besides supplying the nucleophile, has the function of activating the substrate (oligo) by means of its coordination to the free oxygen from the phosphate of the phosphodiester bond that will be hydrolyzed. The Zn1 and Zn3 ions, besides stabilizing the tertiary structure of the nuclease mung bean, bind to the leaving group during the cleavage reaction, impeding that the same reacts with the formed transition state and promotes the reverse reaction. The similarity between K_{d1} (Zn2-mung bean) and K_d (Zn^{2+}-oligo complex) values enable us to enrich the proposed hypothetical model, in the following way: as the oligo approaches the active site of the mung bean nuclease, a displacement of the Zn2 in direction to the phosphodiester bond happens during the formation of the pentacoordinate "transition state". This displacement shows that Zn2 is being shared by mung bean and the oligo, promoting an "yo-yo" effect. Zn2 is displaced in the direction to the phosphodiester bond, exerts its function in the reaction as a catalyst and then comes back to the active site of mung bean nuclease.

REFERENCES

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